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2019-09

Roslund , M , Rantala , S , Oikarinen , S , Puhakka , R , Hui , N , Parajuli , A , Laitinen , O-H , Hyöty , H , Rantalainen , A-L , Sinkkonen , A & The ADELE Research Group 2019 , ' Endocrine disruption and commensal bacteria alteration associated with gaseous and soil PAH contamination among daycare children ' , Environment International , vol. 130 , 104894 . https://doi.org/10.1016/j.envint.2019.06.004

http://hdl.handle.net/10138/304306
https://doi.org/10.1016/j.envint.2019.06.004

Environment International 130 (2019) 104894

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Endocrine disruption and commensal bacteria alteration associated with gaseous and soil PAH contamination among daycare children

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ARTICLE INFO

Handling Editor: Frederic Coulon

ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are priority environmental pollutants that cause adverse health effects. PAHs belong to endocrine signaling disruptors to which children are sensitive to. Recent evidence suggests that PAH pollution alters the abundance of environmental bacteria that is associated with health outcomes. The alteration of environmental and commensal microbiota by PAH pollution has never been connected to endocrine signaling pathways.

To estimate the risk of endocrine disruption in daycare children, we measured PAHs from soil and air of eleven urban daycare centres in Finland. We analyzed daycare yards' soil and children's gut and skin bacterial communities with 16S rRNA gene metabarcoding and used Kyoto Encyclopaedia of Genes and Genomes database to categorize endocrine signaling pathways. We also assessed the PAH hazard to children's health based on the current risk assesments.

We observed associations between signaling pathways in endocrine system and gaseous PAH levels in ambient air. Peroxisome proliferator-activated receptor and adipocytokine signaling pathway decreased with higher chrysene concentration in the air. Soil PAH contamination was associated with altered Actinobacteria, Bacteoridetes and Proteobacteria communities on children's skin and in daycare yard soil. However, adjusted genera were not the same in soil and on skin, with the exception of *Mycobacterium* that was associated with higher PAH concentrations both in soil and on the skin. Even though fluoranhtene levels were above the current threshold values, total PAHs were below safety threshold values and based on current risk assessments there is a minor risk for child health.

Our findings indicate that PAH concentrations that are considered safe may interfere with endocrine signaling by commensal microbiota and alter both environmental and commensal bacterial communities. The imbalance in human microbiota and the decrease in signaling pathways may contribute to emerging public health problems, including inflammatory disorders, obesity and diabetes. Therefore, the optimal risk assessments of PAHs and theoretically also other contaminants shaping commensal microbiota may need to take into account the possibility of the disruption of endocrine signaling pathways.

1. Introduction

Children living in urban areas are exposed to many health-associated environmental stressors. These include increased traffic, air and soil pollution and the loss of natural biodiversity (Kauppi et al., 2012; Parajuli et al., 2017, 2018; Sinkkonen et al., 2013). Comparative

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https://doi.org/10.1016/j.envint.2019.06.004
Received 16 April 2019; Received in revised form 31 May 2019; Accepted 2 June 2019

studies between children living in urban and agricultural environments have shown that commensal microbiota differs and the prevalence of immune mediated diseases is higher among children living in urban environment (Hanski et al., 2012; Kondrashova et al., 2013; Lehtimäki et al., 2017). One of the leading hypothesis is arguing that the core reason behind the high incidence of non-communicable immune-mediated diseases is the loss of natural biodiversity in urban areas (Hanski et al., 2012). This biodiversity loss limits the exposure to diverse microbiota which is essential for the normal development of the immune system. In addition, environmental pollutants, such as polycyclic aromatic hydrocarbons (PAHs) from traffic emissions alter microbial communities in the environment, including those associated with human health (Parajuli et al., 2017; Roslund et al., 2018). As commensal microbiota regulates inflammation and interacts with immune and nervous systems (Blander et al., 2017; Fung et al., 2017), biodiversity loss in parallel with the exposure to pollutants may promote imbalance in human commensal microbiota and increase the risk of immune-mediated disorders.

Polycyclic aromatic hydrocarbons (PAHs) are priority environmental pollutants that are often found in ambient air in agricultural and urban areas (Boström et al., 2002). They are well known carsinogens and mutagens with endocrine distrupting properties that pose a serious threat to human health (Boström et al., 2002). Endocrine distrupting properties of PAHs were first demonstrated in animal models, and then in cell and protein assays (Zhang et al., 2016). A mode of action of endocrine disruption by PAHs involves crosstalk between aryl hydrocarbon receptor (AhR) and peroxisome proliferator-activated receptor (PPAR) in the endocrine system (Borland et al., 2014; Wang et al., 2011). AhR regulates a number of key enzymes involved in PAH metabolism (Beischlag et al., 2008) and PPAR family, including three isotypes (α, β and γ), plays important roles in the regulation of inflammation, cellular differentiation and development, lipid and carbohydrate metabolism, and tumorigenesis (Feige et al., 2006). AhR-mediated disruptions in PPAR pathways affect glucose metabolism and increase the risk of diabetes (Wang et al., 2011).

Numerous studies done with human cells or animal models have connected the PAH exposure and PPAR pathway (Borland et al., 2014; Kim et al., 2005; Wang et al., 2011; Yan et al., 2014) or commensal microbiota and PPAR pathway (Are et al., 2008; Couvigny et al., 2015; Kelly et al., 2004; Nepelska et al., 2017). Importantly, studies linking environmental PAH exposure, human commensal microbiota and PPAR pathway are missing. In this study, we investigated the interference of surface soil PAHs and air gaseous PAHs on children's commensal bacterial community composition and endocrine disrupting functions predicted from metagenome profiles. We specifically focused on PPAR pathway as it is considered as a key pathway linking inflammation and metabolism to the microbiota (Nepelska et al., 2017). Since its signaling cascade includes numerous links, we analyzed two additional endocrine signaling pathways: Adipocytokine and insulin signaling pathways. We sought to find associations between PAH concentrations in daycare center yard's ambient air or surface soils, and on the skin and in the gut microbiota of children. We also assessed the pollution hazard to children's health based on the current risk assessments. We hypothesized that environmental PAHs in daycare environment affect the relative abundance of health-associated commensal bacteria and alter endocrine signaling pathways.

2. Methods

2.1. Experimental setup

Altogether eleven daycare centers participated the study in two cities in southern Finland. Six of them were in Espoo (population over 270,000) located in the Helsinki metropolitan area and five of them were in Lahti (population over 119,000) located about 100 km northeast from the metropolitan area (Fig. 1). The guardians of the children were informed about the research and asked for a signed consent, as advised in the recommendations of the "Finnish Advisory Board on Research Integrity". The ethical statement for the current study was obtained from the local ethical committee (Pirkanmaa Hospital District, Finland). Altogether 53 children, aged between 3 and 5, participated in the study. The children spent five days a week in daycare centers, 8–10 h per day. The use of antibiotics and probiotics was enquired from the children's guardians (Table 1). PAH concentrations were measured from ambient air and surface soils of daycares yards. Bacterial communities were analyzed from soil, stool and skin.

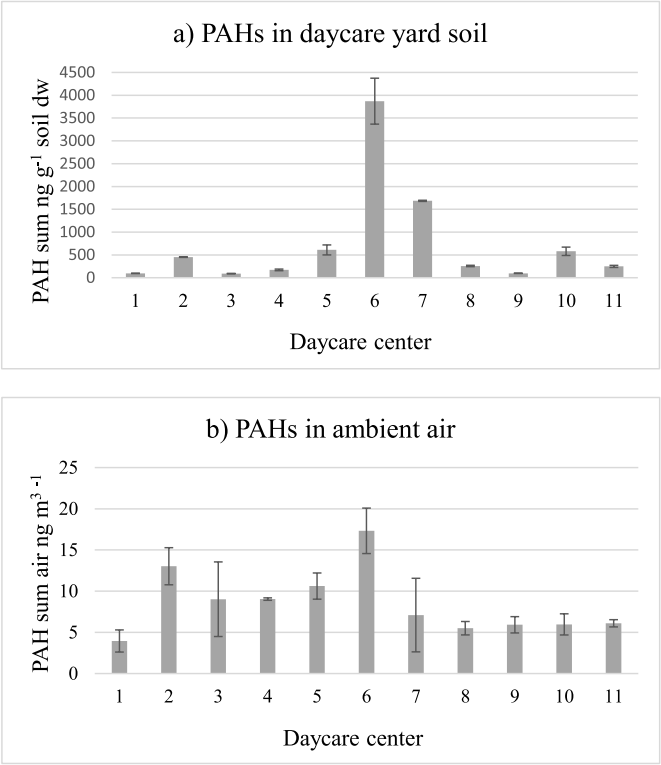

Fig. 1. Polyaromatic hydrocarbon levels a) in soil (ng g^{-1} dry weight) and b) in air (ng m^3/) in eleven daycare center yards in Lahti and Espoo, Finland. Total PAHs were below the Finnish environmental and safety threshold values.

2.2. Soil sampling, hazard assessment and identification of PAH sources

We sampled the uppermost surface soil because children are in daily contact with soil surface; they play with the soil, inhale dust and might lick their mucky hands and ingest the soil. Each yard was sampled on five locations (in front of the main door, in the sand box, on gravel

Table 1
Child information. Total number and sex of participants, and number of skin and stool samples in the study. Antibiotic and probiotic users were removed from gut microbiome tests resulting in 40 children, N = number of children.

	N
Total children	53
Boy	30
Girl	23
Skin samples collected	53
Stool samples collected	47
Excluded (stool analysis):	
Probiotic users	6
Antibiotic users	1

playground, and below the swing and the jungle gym). To assess the hazard to children's health, we used the toxicity equivalent method described in CCME (2010). In short, benzo(a)pyrene total potency equivalent (BaPtpe) for a soil sample was calculated by multiplying the concentration of each potentially carcinogenic PAH in the sample by its BaP potency equivalence factor (PEF) and summing the obtained products. We compared the obtained BaPtpe levels to the incremental lifetime cancer risk (ILCR) for BaP (600 ng g^{-1}) as described in Hiller et al. (2015). This value takes three different pathways into account; direct ingestion of contaminated soil, dermal contact with soil adhering to hands and inhalation of suspended contaminated soil particles. Obtained PAH concentrations were also compared to the Finnish soil quality guidelines (Reinikainen, 2007) (Table S1), which are set according to guidance document on deriving environmental risk limits by European Chemical Bureau (ECB, 2003).

Identification of PAH sources was estimated according to Yunker et al. (2002). This method uses diagnostic ratio of fluoranthene: (fluoranthene + pyrene) (Flt/(Flt + Pyr)), and indeno(1,2,3-*cd*)pyrene: (indeno(1,2,3-*cd*)pyrene + benzo (g,h,i)perylene) (IPy/(IPy + BPe)) as indicators of PAH sources (Table S2).

2.3. Air sampling

We used passive SPMD sampler (SemiPermeable Membrane Device) to measure the concentrations of PAHs in the daycare center yard's ambient air (Huckins et al., 1999). SPMD sampler simulates the process of bioconcentration in the fatty tissues and the samplers are limited to absorb only gaseous PAHs (Bonetta et al., 2009). There were three SPMD samplers in each daycare center yard.

The SPMD samplers were prepared as in Huckins et al. (1999) by cutting the polyethylene tubing into 48 cm long pieces and the strips were soaked twice in n-hexane (liquid chromatography grade from Merck, Germany) for 24 h to remove any potential contaminants. A deuterated fluoranthene was used as a reference compound by mixing 800 μL (10 ng/ μL) Fluoranthene-D10 (10 ng/μL, Dr. Ehrenstorfer GmgH, Augsburg, Germany) with 18.2 g triolein (99% glycerine trioleate, Acros Organics, Belgium). The strips of polyethylene tubing was filled with 520 μL of triolein- fluoranthene -mixture, and the tubing was heat-sealed providing a 45 cm long lipid filled sampler. The samplers were stored in air-tight glass containers in −20 °C until the deployment.

A sampling unit consisted of a SPMD sampler and a 2.5 L zinc container which served as a shelter from rain, wind and solar radiation. Three sampling units per daycare center yard were deployed in front of the main door, and in the center and back of the yard and fastened with a cable tie to a tree, lamppost, downspout or equivalent. The sampling units were placed approximately 2 to 2.5 m above the ground to prevent children touching them and to ensure sufficient air circulation around the unit. Field blanks were included to monitor possible contamination during the deployment of the samplers. After 28-day of sampling, the samplers were collected and frozen at −20 °C individually in glass bottles (50 mL).

2.4. PAH analyzes

The samples were analyzed for 16 EPA (The United States Environmental Protection Agency) priority PAHs. The deuterated PAH mixture (acenaphthene-D10, chrysene-D12, naphthalene-D8, perylene-D12, and phenanthrene-D10) was used as an internal standard (200 ng PAH mix 31, Dr. Ehrenstorfer GmbH, Augsburg, Germany) and anthracene-D10 (100 ng, Dr. Ehrenstorfer GmbH, Augsburg, Germany) as a recovery standard for air and soil samples. All samples were analyzed with gas chromatography–mass spectrometry (Shimadzu GC–MS-QP5000) system equipped with an AOC-20i autoinjector and a 30-m ZB-5MS column (0.25 mm i.d., 0.25 μm film thickness). The mass spectrometer interface temperature was set to 280 °C. The oven temperature program was set as follows: 80 °C for 1 min, 10 °C/min to 250 °C, 7 °C/min to 280 °C and 20 °C/min to final 320 °C for 5 min. One reagent blank was included with each batch of samples being prepared for analysis.

2.4.1. SPMD samplers extraction

Before the PAH analysis SPMD samplers were wiped clean with ethanol to remove any moisture or particulate matter. The samplers were extracted twice by dialysis (24 h) with 50 mL of n-hexane (Merck, Germany) at room temperature and the two extracts of each sample were combined. The samples were first concentrated with a rotary evaporator (Heidolph Laboratory 400) and then further reduced under nitrogen stream approximately to a final volume of 0.5 mL. The aerial concentrations (ng/m^3) of the detected PAHs were calculated using the uptake rates reported by Cranor et al. (2009).

2.4.2. Soil samples extraction

PAH extraction was done as described by Honkonen and Rantalainen (2013) by mixing 1 g soil (wet weight) and 15 mL hexane:acetone mixture (1:1 v/v, Merck Millipore, Darmstadt, Germany).

The extraction was repeated twice by sonicating the samples for 30 min and shaken with 200 rpm for 24 h in the first extraction and for 2 h in the second extraction. The two extracts were combined and concentrated under nitrogen stream to a volume of 1 mL. The extract was cleaned up by using a glass column filled with 1 g of silica gel (Sigma-Aldrich, grade 923, 100–200 mesh) and concentrated under nitrogen stream approximately to a final volume of 0.5 mL. Dry weight was measured after drying the wet soil that was left on the sample tube after extractions in an oven (+105 °C) to constant weight. Soil concentrations are reported as ng g^{-1} dry weight.

2.5. Microbial analyzes

Bacterial communities in soil, stool and on skin were analyzed using Illumina MiSeq 16S rRNA gene metabarcoding with read length 2 × 300 bp using a v3 reagent kit. For skin bacterial community analyzes, we collected swab samples (A cotton wool stick wetted in Tween® 20) from the back of the children's hands (2 × 2 cm area, about 10 s of wiping). Skin swabs were immediately put in dry ice and stored in −70 °C until further processing. Parents collected the stool samples and they were stored in home freezers (−18–20 °C) until the researchers collected them and stored in −70 °C.

Skin and stool samples for MiSeq sequencing were prepared as in Nurminen et al. (2018). Soil samples were prepared as in Parajuli et al. (2018). Soil and stool samples were extracted with PowerSoil® DNA Isolation Kit (Qiagen, Hilden, Germany) and skin swabs with Fast DNA spin kit for soil (MP biomedicals, Santa Ana, CA) according to the manufacturer's standard protocol. The V4 region within the 16S ribosomal RNA (rRNA) gene was amplified by PCR (three replicates from each sample) using 505F and 806R primers (Caporaso et al., 2012). Thus, the primer was identical for soil, stool and skin samples.

Raw sequencing data was processed using Mothur (version 1.39.5, Schloss et al., 2009). The sequence processing protocol partly followed the pipeline suggested by Schloss et al. (2011) and Kozich et al. (2013). The paired sequences contained in reverse and forward fastq files were aligned into a contig. Sequences were trimmed and screened to remove any that had mismatches with primer or DNA-tag sequences, ambiguous bases or homopolymers larger than 8 bp long. Bacterial sequences were aligned against a SILVA reference (version 123, Pruesse et al., 2007), preclustered to minimize sequencing errors (Huse et al., 2010) and screened for chimeras with UCHIME (Edgar et al., 2011). The chimeric sequences were removed and non-chimeric sequences were classified using the Mothur version of Bayesian classifier (Wang et al., 2007) with the RDP training set version 16 (Cole et al., 2009) with 80% bootstrap threshold. Sequences classified to Chloroplast, Mitochondria, unknown, Archaea and Eukaryota were removed from the analyses.

Pairwise distance matrix for unique sequences was calculated and OTUs clustered at 97% sequence similarity using nearest neighbor (single linkage) joining. All bacterial sequence data were accessioned into the Sequence Read Archive (Sequence read Archive Accession SAMN11382605- SAMN11382770). Low abundance OTUs (≤10) were removed from the sequence data. For skin and soil samples, seven OTUs detected in negative controls were removed from sequence data. After removing low abundance OTUs, there were no OTUs detected in stool negative controls.

Samples were subsampled to the smallest sample sequence depth for community composition analyses. Skin samples were subsampled to 4072, stool samples to 450 and soil samples to 6623 sequences. Good's coverage index (average ± SD: Soil 0.99 ± 0.01, stool 0.93 ± 0.02 and skin 1.00 ± 0.00) was used to determine OTU coverage adequacy for diversity and community composition analyzes.

2.6. Prediction of endocrine disruption function

PICRUSt (Langille et al., 2013) was used to generate a profile of putative functions (via metagenome prediction) from the 16S rRNA OTU data classified against the Greengenes Database (Desantis et al., 2006) according to 97% similarity. KEGG (Kyoto Encyclopaedia of Genes and Genomes, release 89.0) database was used to categorize PPAR, adipocytokine and insulin signaling pathways.

2.7. Statistics

All the statistical tests were done with R v3.5.1 (R Development Core Team, 2017) and with *vegan* package. The non-metric multidimensional scaling (NMDS) with Bray-Curtis distance was used to score the bacterial community to the ordination plot. NMDS was done for phyla and classes if the abundance was > 5%, since lower abundances were meaningless in the Bray-Curtis method. The correlation between bacterial community and PAH concentrations were assessed with *envfit* function that fits environmental vectors onto an ordination. Shannon diversity index was determined using the function *diversity* and rarefied species richness using the function *rarefy*. The association between bacterial diversity, richness and relative abundances (> 0.1%), and PAH concentrations were evaluated using generalized linear models (GLM) with quasipoisson distribution at a logarithmic scale. Skin, stool and soil microbial communities were analyzed using all these methods. The input values of PAH concentrations (total PAH, each individual PAH separately and benzo(a)pyrene total potency equivalent) in NMDS and GLM models were yard-averages (either three air measurements or five soil measurements, depending on comparison). Antibiotics and probiotics users were removed from the gut microbiome tests. To conceptualize the false discovery rate (FDR), all the statistical tests were carried out with Benjamini–Hochberg correction (Benjamini and Hochberg, 1995).

3. Results

3.1. Sources and levels of soil pollution

Concentrations of fluoranthene in soil exceeded the allowed environmental threshold level of 1000 ng g^{-1} (Finnish soil quality guidelines) in two daycare centers (Table S3). In a sample taken under a tree in an area characterized by over 50 year old detached houses, BaPtpe was over two times higher (1353 ng g^{-1}) than the incremental lifetime cancer risk level of 600 ng g^{-1}. Another playground soil sample taken from the same yard, the BaPtpe was also higher than the incremental lifetime cancer risk level, being 674 ng g^{-1}. The values of BaPtpe in the other soil samples including the three of five samples taken from the yard with the highest BaPtpe levels, ranged from 0.04 to 233 ng g^{-1} (mean ± SD = 21 ± 55) (Table S3). Total PAH concentrations were below the Finnish environmental and safety threshold values in all samples (< 15,000 ng g^{-1} dw) (Fig. 1, Table S3).

Based on the Flt/(Flt + Pyr) diagnostic ratio, the most common PAH source in daycare center yards was grass, wood and coal combustion, including the yard with the highest BaPtpe and lifetime cancer risk values (Table S3). Based on IPy/(IPy + BPe) diagnostic ratio, almost as often the estimated PAH source was liquid fossil fuel combustion, particularly in one daycare center located in Espoo in the vicinity of a gas station (Table S3).

3.2. PAHs in air are associated with endocrine disruption functions

PPAR (p = 0.04) and adipocytokine signaling pathway (p = 0.05) predicted from the gut metagenome decreased with higher chrysene levels in the air (Fig. 2, Table S4). For skin and soil samples, we did not get reasonable KEGG orthologies i.e. the generalized linear model gave the same statistic values for each pathway. However, there was a correlation between signaling pathways predicted from skin metagenome and chrysene levels in the air (p = 0.05) and phenanthrene levels in the soil (p = 0.01) (Table S4).

3.3. PAHs concentrations are associated with children's commensal microbiota on the skin

The genus *Mycobacterium* (Actinobacteria) was the only taxa that was associated with PAH pollution both in soil and on the skin. The relative abundance of genus *Mycobacterium* on skin increased with higher surface soil levels of PAHs (Fig. S2), and BaPtpe levels in soil (p = 0.01; Fig. 3a and Table S4). In soil, the relative abundance of genus *Mycobacterium* increased with higher fluorene levels (p = 0.004; Fig. 4c and Table S4).

Community composition of bacteria on skin was associated with PAH concentrations in surface soils and in ambient air from OTU to phylum level (p < 0.03, Table S5). Particularly, Actinobacteria and Bacteroidetes community composition on skin was associated with PAH concentrations in soil and in the air (p < 0.01; Table S5). Within Bacteroidetes, the diversity and richness of Class Sphingobacteriia increased with increasing total PAH concentrations in surface soils (p = 0.001; Table S4). In addition, an increase in the relative abundance of two unclassified genera within Proteobacteria and Betaproteobacteria on skin were associated with increasing total PAH concentrations (Fig. S2 and BaPtpe levels in soil (p < 0.005; Fig. 3b and c, Table S4). We did not find associations between gut bacteria and PAH levels (p > 0.50).

3.4. PAHs concentrations are associated with environmental microbiota

In soil, the community composition of the phylum Actinobacteria was associated with PAHs that have five or more benzene rings and with BaPtpe levels (p < 0.02; Table S5). Within Actinobacteria, the relative abundance of genera *Pseudonacardia* (p ≤ 0.002), *Gaiella* (p < 0.001) increased with higher surface soil levels of PAHs with five or more benzene rings and with BaPtpe levels (Fig. 4, Table 2 and Table S4).

In addition, we observed several associations between the community composition (Table S5) or relative abundance (Table S4) of soil bacterial taxa and PAH pollution in soil. At the genus level, the relative abundance of six genera in soil increased with higher total PAH and BaPtpe levels in soil (Table 2).

4. Discussion

This is the first study estimating connections between pollutants, commensal microbiota and signaling pathways that contribute to the inflammation, obesity and diabetes.

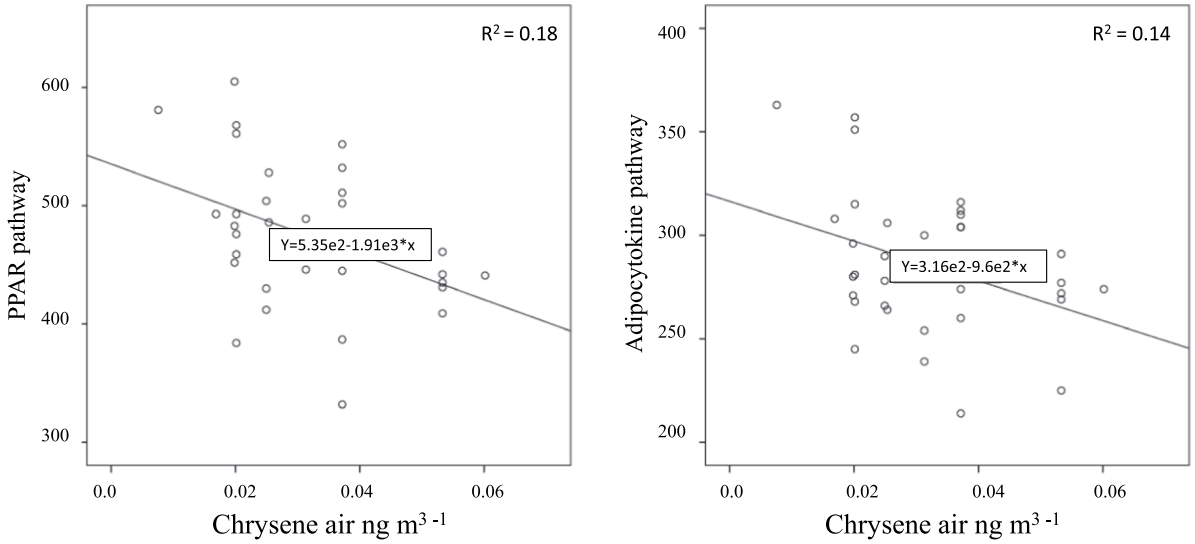

Fig. 2. Air pollution was related to less active endocrine signaling by gut bacteria. Concentrations of chrysene in ambient air were associated with peroxisome proliferator-activated receptor (PPAR) signaling pathway (left) and with adipocytokine signaling pathway (right).

4.1. Endocrine disruption

PPAR and adipocytokine signaling pathway decreased with a higher chrysene concentration in the air, which may increase the risk of several diseases by disrupting hormonally mediated processes (Braun, 2017). Particularly children are sensitive to endocrine disrupting chemicals (Braun, 2017). These processes include glucose metabolism, obesity and insulin sensitivity (Wang et al., 2011), puberty, fertility, thyroid function and carcinogenesis (Street et al., 2018). A previous study has shown consistent results that particularly methylated substitution of chrysene has a strong potency for the disruption of endocrine functions compared to other PAHs (Lee et al., 2017). The epidemic of obesity, diabetes and inflammatory disorders are widely recognized as emerging public health problems, especially in urban societies. Since PPAR signaling pathway regulates inflammation, cell mechanisms, metabolism and tumorigenesis (Feige et al., 2006), the decrease in this pathway may contribute to these emerging public health problems.

Our results connect gaseous PAHs to endocrine disruption. This is understandable as these can easily penetrate from the respiratory system into cells and plausibly to circulation, while PAHs in particulate matter are known to affect lungs and the respiratory tract (Kim et al., 2015). As particles smaller than 1 μm in diameter can potentially end up into circulation as well (Kim et al., 2015), future studies can be designed to study gaseous PAHs and small particulate matter simultaneously. Nevertheless, since gaseous PAHs, rather than particulate PAHs, penetrate into the circulation system and consequently affect gut-brain axis and eventually endocrine system, we were interested in the less studied gaseous PAHs in the air of daycare environments and the associated risks for the health of children.

4.2. Bacteria alterations associated with PAH concentrations

We detected an increased abundance of the genus *Mycobacterium* on children's skin with increasing contamination of PAHs containing four to five benzene rings in the daycare yard soil. In addition, *Mycobacterium* increased in soil with higher fluorene concentrations. Members of the genus *Mycobacterium* have been of great interest in two separate fields in science. First, Mycobacteria belong to the rare microbes that are able to degrade PAHs containing four or more benzene rings which are usually recalcitrant to biodegradation (Kanaly and Harayama, 2000; Kweon et al., 2011; Zeng et al., 2017). Secondly, *Mycobacterium* includes opportunistic pathogens and antibiotic resistant bacteria causing infections in humans and animals (Bottai et al., 2014;

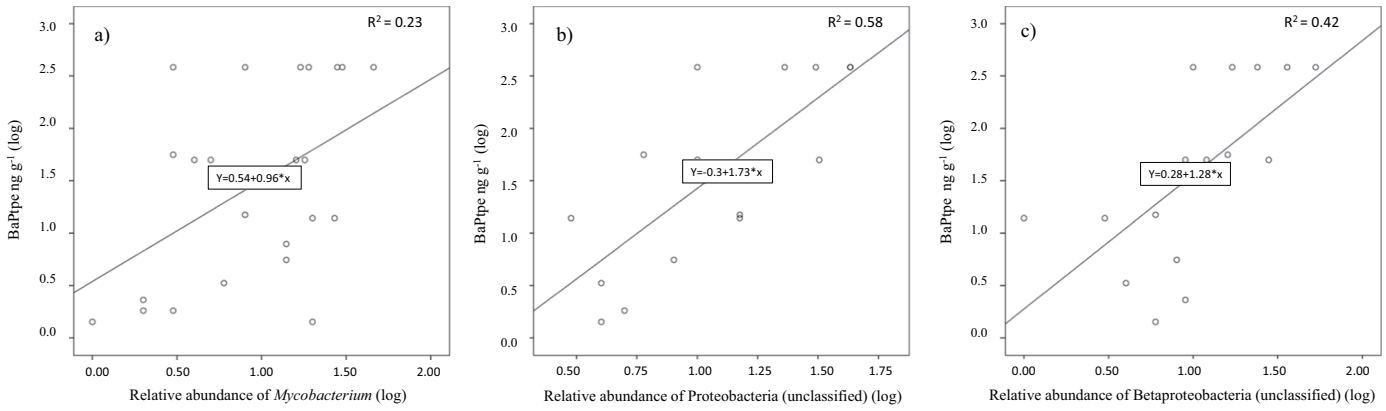

Fig. 3. PAH pollution and relative abundance of skin microbiota
Linear regression of the relative abundance of a) *Mycobacteria*, b) an unclassified genus in Proteobacteria and c) an unclassified genus in Betaproteobacteria on skin against benzo(a)pyrene total potency equivalent (BaPtpe) level in soil.

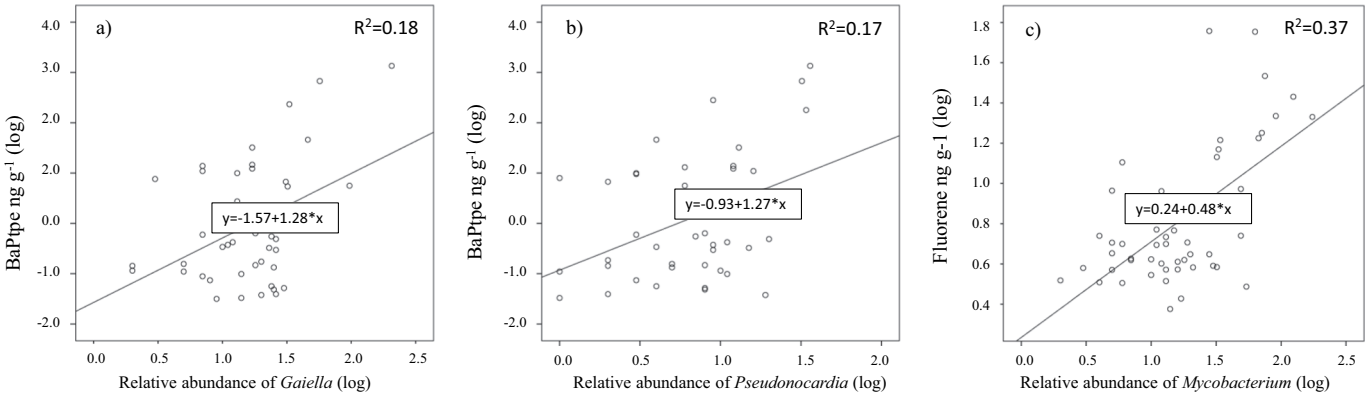

Fig. 4. PAH concentrations in soil affected the relative abundance of three Actinobacterial genera. Associations between a) *Pseudonocardia* and b) *Gaiella* and Benzo[*a*]pyrene total potency equivalent (BaPtpe) level in soil, and between c) *Mycobacterium* and Fluorene concentration in soil.

Table 2
Associations between bacterial relative abundance, and total PAH concentration and Benzo[*a*]pyrene equivalent at the genus level. Generalized linear model showed increase in several bacterial genera with higher total PAH and benzo[*a*]pyrene equivalent concentrations in soil.

Genus	Total PAH level		Benzo[*a*]pyrene equivalent	
	t value	Adjusted p value	t value	Adjusted p value
Terrimonas	4.763	0.001	5.770	< 0.001
Solirubrobacter	4.326	0.002	5.073	< 0.001
Pseudonocardia	3.955	0.004	4.915	< 0.001
Pirellula	3.686	0.008	4.334	0.001
Nitrospira	3.995	0.004	5.023	< 0.001
Gaiella	6.288	< 0.001	7.795	< 0.001
Betaproteobacteria (Unclassified)	4.514	0.001	5.669	< 0.001

Cook, 2010; Howard and Byrd, 2000; King et al., 2017; Primm et al., 2004). In the case of our study, a potential explanation for the observed associations is that pollution-related *Mycobacteria* became more abundant under contamination (Mikkonen et al., 2018).

We also observed an increased abundance of two unclassified genera within Proteobacteria with higher PAH concentrations in the daycare yard soils. Proteobacteria are very often associated with PAH pollution (Mukherjee et al., 2014; Parajuli et al., 2017; Roslund et al., 2018) and the phyla consist also opportunistic human pathogens (Kersters et al., 2006). Thus, our finding leads to a provocative hypothesis that PAHs found from the living environment may alter both environmental and commensal bacterial communities; potentially pathogenic bacteria with degradation ability may become more abundant. Indeed, in our previous study we found that people living in urban areas are more likely to be exposed to *Mycobacterium* and Proteobacterial species, compared to people living in agricultural areas (Parajuli et al., 2018). This might be the effect of PAHs accumulating in urban areas, since in this study the relative abundance of these taxonomies increased with higher PAH concentrations. While *Mycobacteria* includes species that cause serious diseases, for example tuberculosis (*M. tuberculosis*) and leprosy (*M. leprae*), environmental *Mycobacterium* are distinguished from these species by the fact that they are not obligate pathogens (Primm et al., 2004). Actually, this genus includes over 130 species and most of the *Mycobacterium* species are harmless saprophytes (Bottai et al., 2014).

Home environment plausibly contributes to total PAH and bacterial exposure of each children. This is partly due to different environment and partly children's activity patterns. In the studied daycare centers, the daily routines of all children are similar, e.g., children have standardized meals and they exercise in the yards at the same time, several hours a day. Based on our study, probably because children spend approximately 8–10 h per day in daycare, daycare environments have the potential to affect health. To cope with potential negative health associations, daycare yards and other green spaces that are more resilient against PAH pollution should be designed (Roslund et al., 2018). We have previously observed that daily exposure to plant and soil-based materials containing high biodiversity is able to increase the diversity of skin and stool microbiota, and induce regulatory-type immune responses (Hui et al., 2019; Nurminen et al., 2018). These biodiverse materials may be suitable for daycare yards to balance human-induced disturbances in urban environments.

4.3. Risk assessment and sources of PAHs

In one daycare center yard, the BaPtpe was over the protection limit of incremental lifetime cancer risk in two samples. Hence, exposure to these soils might have adverse effects on the health of children playing outdoors. In the same daycare, fluoranthene level exceeded the environmental threshold level, and this threshold was also exceeded in another daycare. However, threshold value means that the concentration is only slightly higher than the background concentration. Adverse health and environmental risks are more likely expected and soil is considered as polluted when the lower standard value exceeds, which is five times higher than the threshold value (Reinikainen, 2007). The lower standard value for PAHs did not exceed in the daycares centers and total PAHs were always under the threshold and safety limits. Therefore, based on the current risk assessments any direct health risk is negligible. However, because PAHs may cause indirect health impacts mediated via commensal bacteria alterations, the current risk assessments may underestimate the hazard of PAHs.

We used diagnostic ratios to estimate PAH sources in daycare yards. Although the environmental fate of PAHs may contribute to the diagnostic ratios over time, the two diagnostic ratios used in this study are the most stable and stated to be the most appropriate for the identification of different PAH sources in soils (Tobiszewski and Namieśnik, 2012). Based on diagnostic ratios, grass, wood and coal combustion was mostly the source of the PAHs. Coal is used as an energy source in Finland, and coal power plant is located in both studied cities. In addition, some daycare centers are located in residential areas where family houses use wood for domestic heating. However, in the larger city, Espoo, the liquid fossil fuel combustion was also a significant source of PAHs. Hence, both coal combustion and vehicular traffic may be significant sources of PAHs for urban soils in the metropolitan area of Finland.

5. Conclusions

Gaseous PAHs in urban ambient air may interfere children's endocrine signaling pathways. We found a decrease in PPAR and adipocytokine signaling pathways with the higher concentration of chrysene in ambient air. The decrease in these signaling pathways may lead to an increased risk of diseases associated with hormonally mediated processes. Our results indicate that PAHs accumulating in daycare yard soils induce shifts both in soil and on children's skin bacterial communities. Particularly, the relative abundance of *Mycobacterium* and two unclassified genera within Proteobacteria on skin increased with higher soil PAH concentrations. These shifts may also include alterations of opportunistic pathogens and shifts may lead to imbalanced human microbiota. Future research should take into account the possibility that environmental PAH contamination alters microbiota both in living environment and in human body, which may affect endocrine signaling pathways that regulate health.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.06.004.

Declaration of Competing Interest

No competing interests.

Acknowledgements

This work was funded by Business Finland (Grant numbers: 6766/31/2017 and 4033/14) and Helsinki Metropolitan Region Urban Research Program (Finland). The authors are very grateful to the ADELE team, Almalab personell, all the daycare centers, children and their families who participated in the study, and the cities of Lahti and Espoo.

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